1: Mol Microbiol. 1998 Jun;28(5):1027-38. Related Articles, Linl

Natural competence for DNA transformation in Helicobacter pylori: identification and genetic characterization of the comB locus.

Hofreuter D, Odenbreit S, Henke G, Haas R.

Max-Planck-Institut fur Biologie, Abteilung, Infektionsbiologie, Tubingen, Germany.

The gram-negative bacterial pathogen Helicobacter pylori, an important aetiological agent of gastroduodenal disease in humans, belongs to a group o bacterial species displaying competence for genetic transformation. Here, we describe the comB gene locus of H. pylori involved in DNA transformation competence. It consists of a cluster of four tandemly arranged genes with partially overlapping open reading frames, orf2, comB1, comB2 and comB3, constituting a single transcriptional unit. Orf2 encodes a 37-amino-acid peptide carrying a signal sequence, whereas comB1, comB2 and comB3 produce 29 kDa, 38 kDa and 42 kDa proteins, respectively, as demonstrated by immunoblotting with specific antisera. For Orf2 and ComB1, no homologous proteins were identified in the database. For ComB3, the best homologies were found with TraS/TraB from the Pseudomonas aeruginosa conjugative plasmid RP1 and TrbI of plasmid RP4, VirB10 from the Ti plasmid of Agrobacterium tumefaciens and PtlG, a protein involved in secretion of pertussis toxin of Bordetella pertussis. Defined transposon knocl out mutants in individual comB genes resulted in transformation-defective phenotypes ranging from a 90% reduction to a complete loss of the natural transformation efficiency. The comB2 and comB3 genes show homology to HP0528 and HP0527, respectively, located on the cagII pathogenicity island of H. pylori strain 26695.

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1: J Tongji Med Univ. 2000;20(4):273-6. Related Articles, Linl

Multiple defects of cell cycle checkpoints in U937-ASPI3K, an U937 cell mutant stably expressing anti-sense ATM gene cDNA.

Zhou J, Liu W, Sun L, Sun H, Tang Y.

Department of Hematology, Tongji Hospital, Tongji Medical University, Wuhan 430030.

(Ataxia-telangiectasia mutated gene (ATM) functions in control of cell cycle checkpoints in responding to DNA damage and protects cells from undergoing apoptosis. Knock-out within tumor cells of endogenous ATM wi achieve therapeutic benefits and enable a better understanding of the decisive mechanisms of cell death or survival in response to DNA damaging agents.) In present paper, we sought to characterize the cell cycle checkpoint profiles in U937-ASPI3K, a U937 cell mutant that was previously established with endogenous ATM knock-out phenotype. Synchronized U937-ASPI3K was exposed to 137Cs irradiation, G1, S, G2/M cell cycle checkpoint profiles were evaluated by determining cell cycle kinetics, p53/p21 protein, cyclin dependent kinase 2 (CDK2) and p34CDC2 kinase activity in response to irradiation. U937-ASPI3K exhibited multiple defects in cell cycle checkpoin as defined by failing to arrest cells upon irradiation. The accumulation of cellular p53/p21 protein and inhibition of CDK kinase was also abolished in U937-ASPI3K. It was concluded that the stable expression of anti-sense PI3I cDNA fragment completely abolished multiple cell cycle checkpoints in U937-ASPI3K, and hence U937-ASPI3K with an AT-like phenotype could serves as a valuable model system for investigating the signal transduction pathway in responding to DNA damaging-based cancer therapy.

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1: J Bacteriol. 2000 Feb;182(4):937-43.

A set of genes encoding a second toluene efflux system in Pseudomonas putida DOT-T1E is linked to the tod genes for toluene metabolism.

Mosqueda G, Ramos JL.

Department of Biochemistry and Molecular Biology of Plants, Estacion Experimental del Zaidin, Consejo Superior de Investigaciones Cientificas, E-18008 Granada, Spain.

Sequence analysis in Pseudomonas putida DOT-T1E revealed a second toluene efflux system for toluene metabolism encoded by the ttgDEF genes, which are adjacent to the tod genes. The ttgDEF genes were expressed in response to the presence of aromatic hydrocarbons such as toluene and styrene in the culture medium. To characterize the contribution of the TtgDE system to toluene tolerance in P. putida, site-directed mutagenesis was used t knock out the gene in the wild-type DOT-T1E strain and in a mutant derivative, DOT-T1E-18. This mutant carried a Tn5 insertion in the ttgABC gene cluster, which encodes a toluene efflux pump that is synthesized constitutively. For site-directed mutagenesis, a cassette to knock out the ttgD gene and encoding resistance to tellurite was constructed in vitro and transferred to the corresponding host chromosome via the suicide plasmid pKNG101. Successful replacement of the wild-type sequences with the mutant cassette was confirmed by Southern hybridization. A single ttgD mutant, DOT-T1E-1, and a double mutant with knock outs in the ttgD and ttgA genes, DOT-T1E-82, were obtained and characterized for toluene tolerance. This was assayed by the sudden addition of toluene (0.3% [vol/vol]) to the liquid culture medium of cells growing on Luria-Bertani (LE medium (noninduced) or on LB medium with toluene supplied via the gas phase (induced). Induced cells of the single ttgD mutant were more sensitive to sudden toluene shock than were the wild-type cells; however, noninduced wild-type and ttgD mutant cells were equally tolerant to toluene shock. Noninduced cells of the double DOT-T1E-82 mutant did not survive upon sudden toluene shock; however, they still remained viable upon sudden toluene shock if they had been previously induced. These results are discussed in the context of the use of multiple efflux pumps involved in solvent tolerance in P. putida DOT-T1E.